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ANOPHELES (CELLIA) MINIMUS THEOBALD (DIPTERA: CULICIDAE): NEOTYPE DESIGNATION, CHARACTERIZATION, AND SYSTEMATICS

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Abstract.—A neotype for *Anopheles* (*Cellia*) *minimus* Theobald, the nominotypical member of a malaria vector species complex in the Oriental Region, is designated from a series of nine individually reared specimens collected at Ham Hang Mei, New Territories, Hong Kong, China. A hindleg taken from each of the specimens was used for DNA extraction. The specimens were identified as species A of the complex based on sequences for the third domain (D3) of the 28S rDNA locus and the mitochondrial cytochrome oxidase subunit II locus (COII). The species is described from the neotype series, the larval and pupal stages of the neotype are illustrated, and sequence data are provided for the D3 and COII loci.

Key Words: Anophelinae, D3 sequence, COII sequence, mosquito, taxonomy

Anopheles minimus Theobald is widely distributed in hilly areas throughout much of the Oriental Region, extending northward to about 32°30′N in China, westward to Uttar Pradesh in India, southward through peninsular Malaysia and eastward to the Ryukyu Archipelago of Japan. It is regarded as an important vector of human malaria throughout its distribution (Ho and Feng 1958, Reid 1968, Harrison 1980, Harrison et al. 1991, Chen et al. 2002). Green et al. (1990) showed that *An. minimus* consists of two species (denoted as species A and C) in western Thailand based on the sympatric occurrence of homozygotes of two enzyme loci in the absence of heterozygotes. Green and colleagues (see Baimai 1989) also recognized a third species (denoted as species D) in sympatry with species A and C based on electrophoretic data. Sharpe et al. (1999, 2000) confirmed the presence of species A and C in western Thailand and suggested the possible presence of a third species (specimen #157). Chen et al. (2002) concluded that forms A and B of Yu and Li (1984) and Yu (1987) in China are morphological variants of species A, and that subspecies x of Baba (1950) in southern China probably refers to *An. aconitus* Dönitz. Recently, Somboon et al. (2001, 2005) provided morphological, cytogenetic, molecular, and hybridization evidence for the recognition of another sibling species of the Minimus Complex on Ishigaki Island, Japan, which they informally designated as species E.

Anopheles minimus species A appears to be the predominant species of the Minimus

Complex in the Oriental Region. It is recorded from northeastern India to eastern China and southward from Sichuan Province of China through Laos, Thailand, Vietnam, and Cambodia (Subbarao 1998; Van Bortel et al. 1999, 2000; Kengne et al. 2001; Chen et al. 2002). Sharpe et al. (2000) estimated that the long-term effective population size of species C should be approximately half that of species A. This is concordant with its smaller range: it is only recorded from western and northern Thailand (Green et al. 1990, Sharpe et al. 1999), northern Vietnam (Van Bortel et al. 1999, 2000; Kengne et al. 2001), south-central provinces of China (Chen et al. 2002), and northern areas of India (Chen et al. 2006). In China, species A occurs in sympatry with species C in Yunnan and Guangxi Provinces and extends eastward and southward into Guangdong, Hainan, and Taiwan Provinces.

Anopheles minimus was named and described by Theobald (1901) from a single female collected in Pokfulam, Hong Kong, but the specimen no longer exists (Theobald 1910, Yamada 1925, Harrison 1980). Harrison (1980) stated: "If a neotype is ever needed, numerous adults with associated immature skins collected only 20–22 km from Pokfulam (Hong Kong Island) in Sai Kung District, New Territories, are deposited in the USNM." Sharpe (1997) examined some of those specimens but was unable to determine whether they were representatives of species A or C. She attempted to sequence the D3 region of 28S rDNA from several specimens but the sequences turned out to be those of a yeast-like organism rather than a mosquito. Because species A is known to have a much wider distribution than species C, which has not been found in eastern China, it has been suggested that the former is likely to be conspecific with the species originally described as *An. minimus* from Hong Kong (Harrison et al. 1991). However, before the name of *minimus* can be unambiguously assigned to species A, a neotype unequivocally identified as species A from as near as practicable to the original type locality must be designated to fix the application of the name. Consequently, the purpose of this paper is to fix the identity of *An. minimus s.s.* as a foundation for further studies and the formal taxonomic recognition of other species of the complex.

Materials and Methods

This study is based on nine adult mosquitoes, with associated larval and pupal exuviae, that were reared from fourth-instar larvae collected at Ham Hang Mei, New Territories, Hong Kong, China (22°13′N, 114°13′E) by Leopoldo Rueda and James Pecor of the Walter Reed Army Institute of Research, Washington, DC. Observations of adults were made under simulated natural light. Larval and pupal chaetotaxy were studied using differential interference contrast microscopy. Measurements and counts were taken from all specimens. Unless indicated otherwise, numbers in parentheses represent modes of the reported ranges. The morphological terminology used in the species description follows Harbach and Knight (1980, 1982). The specimens are deposited in the National Museum of Natural History (NHNH), Smithsonian Institution, Washington, DC.

A hindleg was removed from each of the nine adults for DNA extraction. DNA was extracted individually from each leg using the procedure developed by Linton et al. (2001) and amplified with the PCR primers for part of the mitochondrial COII gene and the nuclear 28S ribosomal DNA D3 region used by Chen et al. (2002). Products were sequenced on an Applied Biosystems ABI377 using the PCR primers and manufacturer's protocols.

Taxonomic Treatment

Anopheles (*Cellia*) *minimus* Theobald

1901. *Anopheles minimus* Theobald, 1901: 186 (published 23 November). Neotype (hereby designated), female (CH6–3)

with associated fourth-instar larval and pupal exuviae on microscope slide: CHINA, Hong Kong, New Territories, Ham Hang Mei (NMNH).

1901. *Anopheles vincenti* Laveran, 1901: 993 (published 29 November). Syntypes, 5 females: VIETNAM, Tonkin, Van-Linh (PIP).

1902. *Anopheles formosaensis I* Tsuzuki, 1902: 288. Lectotype, female: TAIWAN (NHM); designation by Harrison (1980).

1902. *Anopheles christophersi* Theobald, 1902: 378. Lectotype, female: INDIA, [West Bengal], Duars (NHM); designation by Harrison (1980).

1903. *Anopheles aconitus* var. *cohaesa* Dönitz, 1903: 233 (*nomen novum* for *formosaensis I* Tsuzuki, Sep 1902, *non formosaensis II* Tsuzuki, Feb 1902). Unjustified replacement name (see Harrison 1980: 83).

1910. *Myzomyia christophersi* var. *alboapicalis* Theobald, 1910: 25. Holotype, female: INDIA, [West Bengal], Duars, Jalpaiguri, Meenglas (NHM).

Anopheles minimus form A of Yu and Li 1984 (morphology); Yu 1987 (morphology).

Anopheles minimus form B of Yu and Li 1984 (morphology); Yu 1987 (morphology).

Anopheles minimus species A of Green et al. 1990 (enzyme electrophoresis, morphology); Baimai et al. 1996 (mitotic karyotype); Sucharit and Komalamisra 1997 (RAPD-PCR identification); Sharpe et al. 1999 (D3 rDNA, ASA and SSCP identification); Sharpe et al. 2000 (COII mtDNA, ITS2 rDNA, D3 rDNA, phylogenetic relationships); Van Bortel et al. 2000 (ITS2 rDNA, SCAR multiplex assay); Kengne et al. 2001 (RAPD-PCR assay); Somboon et al. 2001 (D3 rDNA, crossmating); Chen et al. 2002 (D3 rDNA, morphology, distribution); Choochote et al. 2002 (crossmating); Zhou et al. 2002 (COII mtDNA, phylogenetic relationships); Chen et al. 2003 (COII mtDNA, D3 rDNA, phylogenetic relationships); Phuc et al. 2003 (ITS2 rDNA, multiplex assay); Van Bortel et al. 2003 (*Odh* locus, population genetics); Garros et al. 2004 (ITS2 rDNA, single multiplex assay); Garros et al. 2005a (COI mtDNA, ITS2 rDNA, D3 rDNA, phylogenetic relationships); Garros et al. 2005b (COII mtDNA, D3 rDNA, morphology, phylogenetic relationships).

Anopheles minimus form I of Van Bortel et al. 1999 (*Odh* locus).

Diagnosis.—Sequences for the D3 domain of the 28S rDNA locus and the mitochondrial cytochrome oxidase subunit II locus (COII) for *An. minimus,* other members of the Minimus Complex, and related species of the Myzomyia Series show no intraspecific variation, and thus represent species-diagnostic characters (see below).

Female.—*Head:* Vertex with patch of pale (white to yellowish) erect scales behind frontal tuft, dark (brown to black) erect scales laterally and posteriorly; interocular space with some white falcate scales, several long golden-brown setae and frontal tuft of long white sinuous setae. Clypeus bare. Antenna length about 1.1 mm; pedicel with pale integument and few minute setae on mesal and lateral surfaces; flagellomeres 1–3 with pale scales on mesal surfaces. Proboscis about 1.5 mm, slightly longer than forefemur; prementum entirely dark-scaled, scales appressed throughout except for few slightly erect scales at base; labella pale. Maxillary palpus 1.4–1.6 mm long, with 3 pale bands (in dorsal view), apical and preapical pale bands about length of preapical dark band, narrow pale band at apex of palpomere 2; palpomere 2 with semi-erect scales giving bushy appearance to proximal portion of palpus; ventral surface of palpus without scales. *Thorax:* Integument brown, pleura with darker transverse areas; scutum with broad median pale pruinose area confluent with scutellum of similar appearance; anterior promontory and antedorsocentral areas with long erect white falcate scales that abruptly grade into decumbent golden

piliform scales on acrostichal and dorsocentral areas that extend posteriorly to and then on lateral margins of prescutellar area to scutellum; long golden to golden-brown setae on acrostichal, dorsocentral and prescutal areas, dark setae on fossal, antealar and supraalar areas. Scutellum with row of golden piliform scales adjacent to posterior row of long bronze to brown setae. Mesopostnotum and postpronotum bare. Antepronotum without scales, with long dark setae. Pleura with brown to golden setae on upper proepisternum (1), prespiracular area (0–2), prealar knob (2–5), upper (2,3) and lower (3) mesokatepisternum and upper mesepimeron (4–7). *Wing:* Length about 2.7–3.0 mm; dark scaling black, stark on costa, subcosta and R–R_1, subdued on posterior veins, pale scaling pale yellow, not white; costa with presector pale, sector pale and preapical pale spots, remigium and base of radius pale to presector dark spot, sector and accessory sector pale spots of R fused or separated by small dark spot; R_s and R_{2+3} dark in middle, R_2 and R_3 pale at origin, and R_2 also pale at apex; R_{4+5} with postbasal and preapical dark spots; M_{1+2}, M_1, M_2, M_{3+4} and CuP pale at base and apex; M_1 with short and M_{3+4} with long middle pale spot; mcu dark-scaled; CuP with moderately long postbasal and preapical dark spots and small dark spot at junction of mcu; 1A with pale base followed by postbasal dark and pale spots of about same length, about distal 0.5 dark-scaled; pale fringe spots at apices of CuP, M_{3+4}, M_2, M_1, R_{4+5}, R_3 and from R_2 to slightly anterior to R_1 (total of 7 pale fringe spots). *Halter:* Pedicel pale, scabellum and capitellum dark, capitellum dark-scaled. *Legs:* Coxae and trochanters without scales; femora, tibiae and tarsi dark-scaled, apices of tibiae indistinctly pale, tarsomeres 1–4 with minute faint dorsoapical pale spots. *Abdomen:* Integument dark with uniform covering of golden setae, without scales.

Male.—Similar to female except for obvious sexual differences; other differences include the following. *Head:* Eyes more widely separated, decumbent falcate scales slightly more numerous. Proboscis longer and more slender, about 1.9 mm, approximately 1.4 length of forefemur. Maxillary palpus largely dark-scaled, with pale scaling as follows: narrow dorsolateral patch at apex of palpomere 3, large dorsolateral patch closer to anterior than to posterior margin of palpomere 4, and covering entire dorsolateral surface of palpomere 5. *Wing:* Generally paler and scaling reduced, fringe spots less distinct. *Genitalia:* Not dissected, see Harrison (1980: Fig. 16).

Pupa (Figs. 1A, B).—Character and positions of setae as figured; numbers of branches in Table 1. *Cephalothorax:* Lightly and more or less evenly pigmented, appendages and metanotum with darker areas. Seta 7-CT long, about twice length of 6-CT, usually double, sometimes triple; 8-CT single; 10-CT with 1–3(2) branches. *Trumpet:* Moderately pigmented; length 0.24–0.40 mm (mean = 0.37 mm); meatus very short, 0.04–0.10 mm (mean = 0.07 mm); pinna long, 0.25–0.33 mm (mean = 0.29 mm). *Abdomen:* Length 2.09–2.53 mm (mean = 2.29 mm); lightly pigmented, anterior margins of more anterior segments darker; terga and sterna minutely spiculate. Seta 0-III–VII long, usually with 2,3 branches (1–4), inserted anterior to seta 2 on segment III, far laterad of seta 2 on segments IV–VII, more or less directly anterior to seta 5 on segments V–VII; seta 1-II–VI with multiple thin flexible branches, 1-V,VI usually and 1-VII always single, long, exceeding length of following tergum; 6-I,II long, more than twice length of seta 7, 6-I often double (single to 6-branched), 6-II single; 6-III–V always and 6-VI,VII usually branched, number of branches generally progressively decrease from 6-III to 6-VII; seta 7-III–V short, normally branched, 7-VI,VII single, long, about length of following sternum, 7-III–VI inserted on lateral side of fold line before posterior margin of segment, 7-VII inserted on fold line at posterior margin of segment; setae 8,10,11-II usually absent, on one side when present,

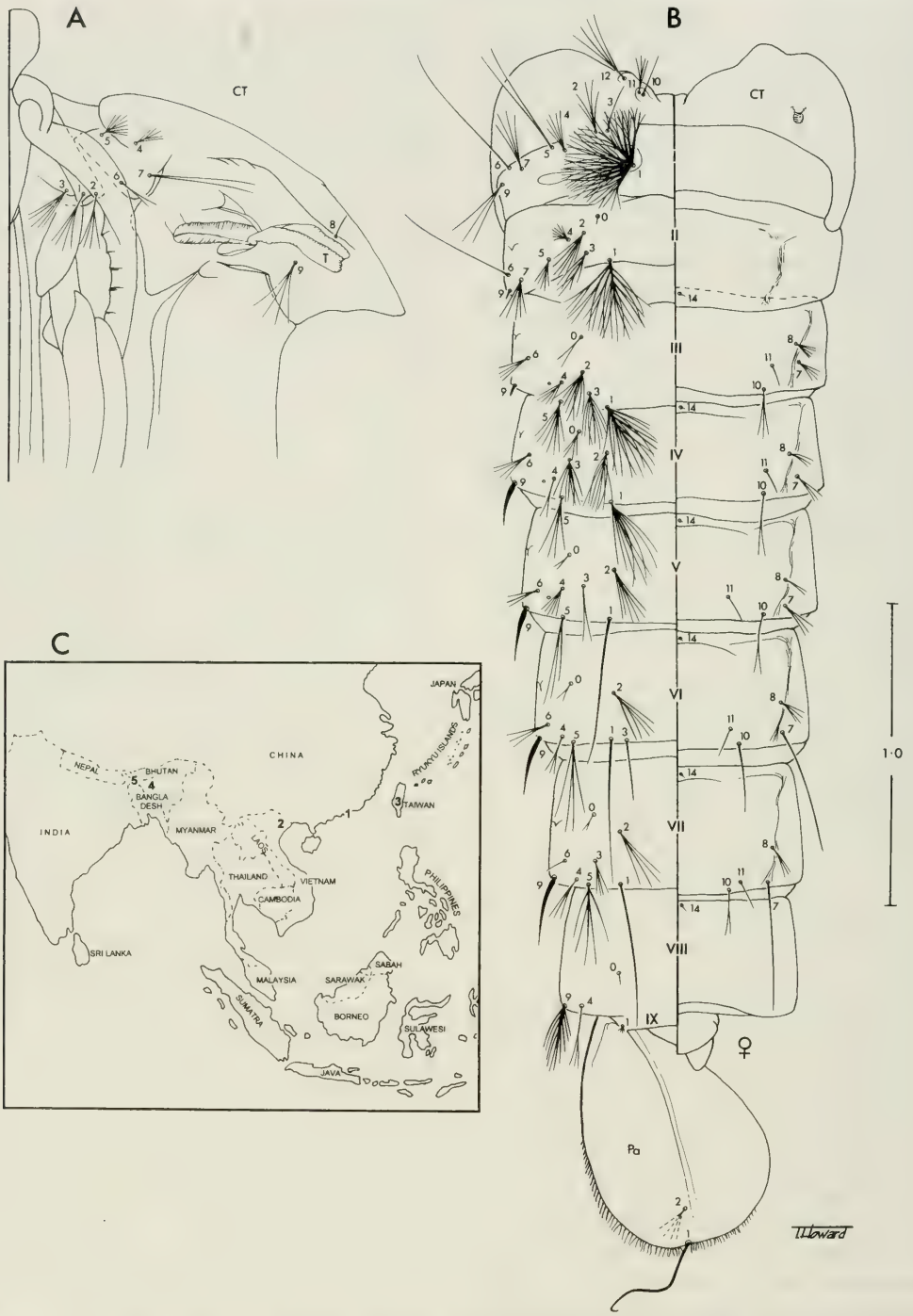

Fig. 1. A,B, Pupa of *Anopheles minimus*, modelled after exuviae of neotype female. A few missing setae were drawn from a paraneotype. A, Left side of cephalothorax, dorsal to right. B, Dorsal (left) and ventral (right) aspects of metathorax and abdomen. C, Map of southern Asia showing the type localities (see text for names) of *An. minimus* and its synonyms (1, *minimus*; 2, *vincenti*; 3, *formoasensis I*; 4, *christophersi*; 5, *alboapicalis*). CT = cephalothorax; Pa = paddle; T = trumpet; I–IX = abdominal segments I–IX; 0–14 = setal numbers for specified areas, e.g., seta 3-I. Scales in mm.

Table 1. Range (mode, where apparent) of numbers of branches for pupal setae of the neotype series of *Anopheles minimus*.

Setae No.	Cephalothorax CT	Abdominal Segments I	II	III	IV	V	VI	VII	VIII	IX	Paddle P
0	—	—	1	1–4 (2)	2–4 (3)	1–3 (2)	1–3	1–4 (2)	1	—	—
1	3–5 (4)	nc	16–32 (22)	12–21	7–13 (9)	1–3 (1)	1, 2 (1)	1	—	2–4 (3)	1
2	2–5 (4)	5–5 (5)	4–8 (6)	6–11 (7)	5–9 (7)	4–7 (6)	3–6 (5)	3-5 (4)	—	—	2–5 (3)
3	4–6 (5)	1–3 (2)	5–8 (5)	5–10 (8)	6–8 (7)	1–5 (3)	1–3 (1)	2–4 (3)	—	—	—
4	1–3 (3)	5–9	3–7 (4)	3–5 (5)	2, 3 (2)	2–5 (3)	1–3 (3)	1–3 (2)	1–3 (2)	—	—
5	6–11 (8)	2–4 (3)	4–8 (7)	7–12 (10)	3–9 (7)	3–7	3–7 (5)	3–5 (4)	—	—	—
6	2–6 (3)	1–6 (2)	1	2–7 (5)	3–6 (4)	2–4 (3)	1–3 (2)	1–3 (1)	—	—	—
7	2, 3 (2)	4–6 (4)	3–8 (4)	2–6 (5)	1–5	2–6 (4)	1	1	—	—	—
8	1	—	0–2 (0)	2–5 (4)	2, 3 (3)	1–3 (2)	2–4 (2)	1–6 (3)	—	—	—
9	3–6 (4)	2–5 (3)	1	1	1	1	1	1	8–12 (9)	—	—
10	1–3 (2)	—	0, a, 2	2–4 (4)	1–3 (2)	1–3 (1)	1–3 (2)	1–4 (2)	—	—	—
11	3–6 (5)	—	0, 1 (0)	1	1	1	1	1, 2 (2)	—	—	—
12	3–7 (4)	—	—	—	—	—	—	—	—	—	—
14	—	—	—	1	1	1	1	1	1	—	—

nc = not counted; a = alveolus only.

8-II usually double (1,2), 10-II represented by alveolus in 3 specimens and double seta in one specimen, 11-II single when present; seta 9-I relatively long, about half length of 6-I, with 3–6(4) branches; 9-II,III small, peglike; 9-IV–VII long, curved, simple and sharply pointed, length progressively increasing from about 0.4 of segment IV to 0.6 of segment VII; 9-VIII slightly shorter than 9-VII, about length of 4-VIII, plumose with 9–14(10) close-set branches arising from broad flattened central stem; 10-II absent. *Genital lobe:* Length 0.16–0.18 mm in female; 0.33–0.37 mm in male, with nipple at apex. *Paddle:* Lightly pigmented (hyaline), buttress and midrib slightly darker, midrib distinct to near seta 2-Pa; length 0.61–0.70 mm (mean = 0.65 mm), width 0.40–0.46 mm (mean = 0.43 mm), index 1.4–1.65 (mean = 1.53); marginal serrations begin 0.28–0.35 from base and end 0.47–0.54 from base where they are replaced abruptly by short hyaline filaments; refractile index 0.27–0.33 (mean = 0.30). Seta 1-Pa long, sinuous, with hooked tip, about one-third length of paddle, arising from shallow emargination at apex.

Larva, fourth-instar (Fig. 2).—Character and positions of setae as figured; numbers of branches in Table 2. *Head:* Slightly wider than long, width 0.57–0.66 mm (mean = 0.61 mm), length 0.54–0.60 mm (mean = 0.57 mm); integument with mottled pattern of moderately to darkly pigmented areas, with some intervening lightly pigmented areas; collar and mentum darkly pigmented. Setae 2,3-C single, simple; 3-C 0.5–0.6 length of 2-C; 4-C single, reaching just beyond alveolus of 2-C, inserted in line with 5-C; 8-C usually dendritic, with 4–7(6) branches; 13-C inserted posterolateral to 11-C. *Antenna:* Moderately to darkly pigmented; strong barb-like spicules on mesal surface, weaker ones on ventral surface; length 0.20–0.24 mm (mean = 0.21 mm). Seta 1-A short, single, simple, length about diameter of antenna, inserted about 0.3 from base on outer dorsolateral surface; 4-A with 4–7(5) branches. *Thorax:* Integu-

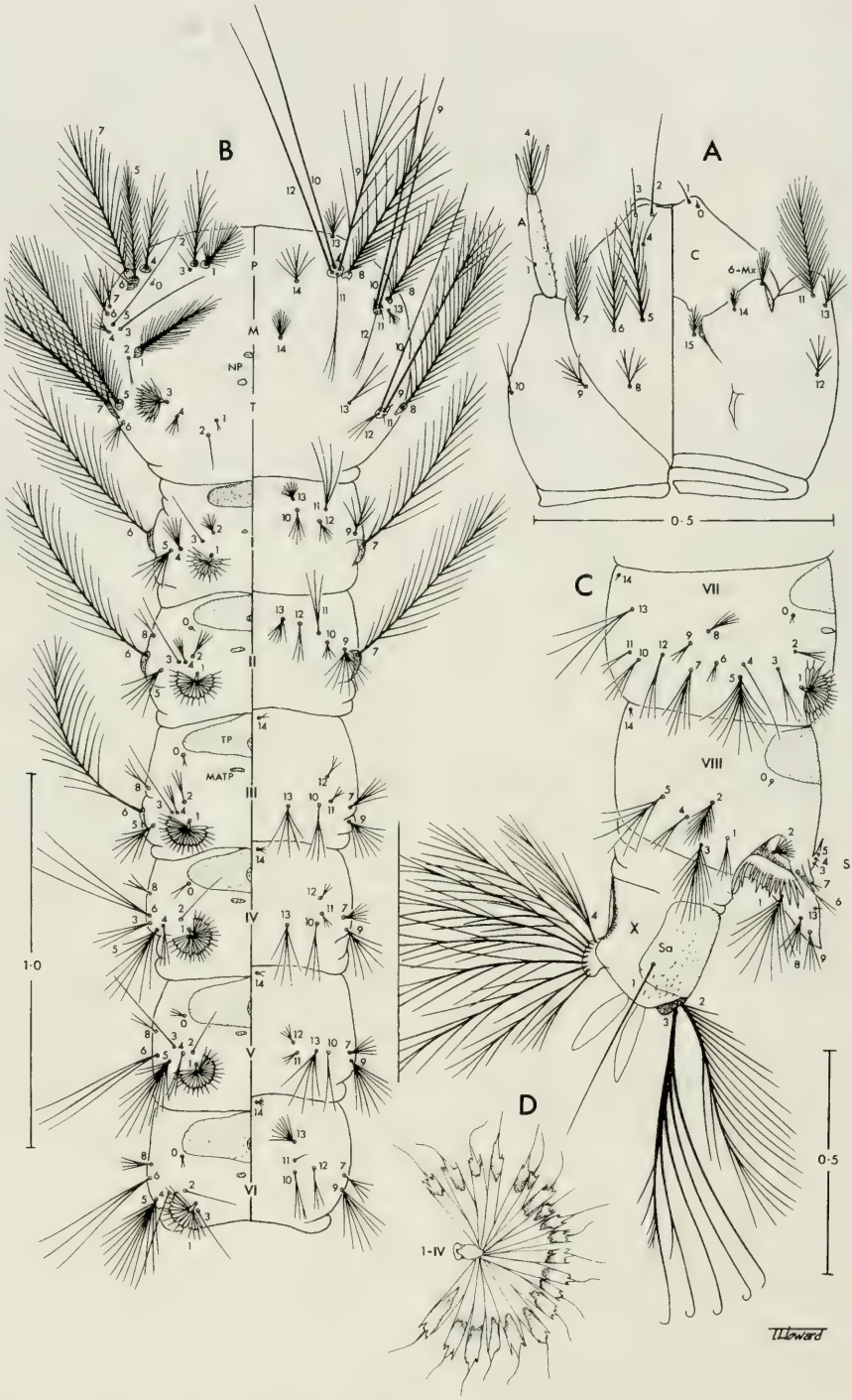

Fig. 2. Fourth-instar larva of *Anopheles minimus*, reconstructed from exuviae of neotype female. A few setae missing on the right side of the exuviae were drawn from the left side, and vice versa. A, Head, dorsal (left) and ventral (right) aspects of left side. B, Thorax and abdominal segments I–VI, dorsal (left) and ventral (right) aspects of left side. C, Abdominal segments VII–X, left side. D, Seta 1-IV. A = antenna; C = cranium; M = mesothorax; MATP = median accessory tergal plate; NP = notal plate; P = prothorax; S = spiracular lobe; Sa = saddle; T = metathorax; TP = tergal plate; I–VIII,X = abdominal segments I–VIII,X; 0–15 = setal numbers for specified areas, e.g., seta 5-C. Scales in mm.

Table 2. Range (mode, where apparent) of numbers of branches for fourth-instar larval setae of the neotype series of *Anopheles minimus*.

Setae No.	Head	Thorax			Abdominal Segments								
	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	1	1	–	–	–	1	1,2(2)	2,3(2)	2,3(2)	1,2(2)	1,2(2)	1,2(1)	–
1	1	19–29	25–37(28)	1,2(2)	11–16(13)	13–20(18)	16–22(21)	17–23	16–21(18)	15–21(17)	15–19(16)	1–4	1
2	1	13–17(14)	1,2(1)	1	4–7(5)	3–6(5)	2–4	1	1	1,2(1)	3–5(3)	7–14(13)	19–22(19)
3	1	1,2(1)	1,2(1)	11–18(16)	1	1	1	2–4(2)	1	1	3	8–13(11)	8–13
4	1	8–16	3–6	3–5(4)	4–7(6)	4–7(5)	2–5(3)	2–5(4)	2,3(3)	1	1	3–5(3)	4–13*
5	11–16(15)	27–42(28)	1	32–41(35)	5–8(6)	5–8(5)	6–10(6)	6–10	8–13(10)	9–13(11)	10–13(11)	4–6(5)	–
6	12–17(14)	1	3–6(4)	3–5(4)	25–31(29)	23–31(25)	16–24	3	3	3,4(3)	3–6(3)	–	–
7	14–18(16)	24–30(26)	3,4(3)	29–39	22–33(28)	24–32	4–8(5)	5–7(6)	4–8(5)	3–5(4)	3–7(5)	1-S,	7–11
8	4–7(6)	31–34(34)	16–26(23)	27–39(34)	–	2,3(3)	2,3(2)	2–4(2)	1–3(2)	2–4(2)	3–8(5)	2-S,	7–10(8)
9	3–7	9–13(10)	1	7–9(7)	5,6(6)	7–9(7)	5–8(7)	4–7(6)	5–8(7)	7–10(8)	3–10(8)	6-S,	2–4(3)
10	2,3(3)	1	1	1	2–5(3)	2–4(3)	2–4(3)	2,3(3)	2,3(3)	3	4–7(5)	7-S,	1,2(2)
11	30–44	2–5(3)	1	1	3– 5(4)	2,3(3)	2–4(3)	2–5(3)	2–4(3)	2–4(3)	2,3(2)	8-S,	4–7
12	4–7(6)	1	1,2	3– 5(3)	3–5(4)	2– 5(3)	1–4(3)	2–4	2–4(3)	2–4(3)	1–3(3)	9-S,	4–6(4)
13	5–9(7)	4–7(5)	5–9	3,4(3)	4–9(8)	6– 10(8)	5–11	4– 6(6)	3–5	6–11(8)	3– 5(3)	–	–
14	5–7	3– 5(5)	8–12	–	–	–	1–4(2)	1–4(3)	2–4(2)	2–5(2)	1–3(1)	2	–
15	6–9	–	–	–	–	–	–	–	–	–	–	–	–

*Range of branches for individual setae (9 pairs).

ment hyaline, smooth. Mesothorax with conjoined pair of median notal plates, sometimes also with pair of submedian notal plates; metathorax usually with separated pair of notal plates, apparently without submedian notal plates. Setae 1,2-P inserted on narrowly separated tubercles; support plate of pleural setal groups 9–12-P,M,T with short spine; 9-P,T relatively sparsely plumose, 9-P with 9–13(10) branches, 9-T with 7–9(7) branches usually all arising from distal half of rachis; 10-P, 9,10-M and 10-T long, single, simple; 11-P large, significantly larger than 11-M,T, divided distally into 2–5(3) branches. Seta 4-M with 3–6 branches arising at base; 12-M single or double, branched at midlength. Seta 3-T with short thick stem bearing 11–18(16) lanceolate leaflets; 12-T shorter than 12-M, with 3–5(3) branches from near base. *Abdomen:* Integument hyaline, smooth; tergal plates of segments II–VIII very large, about 0.6–0.7 width of segment, enclosing small median accessory tergal plate (not always enclosed on segment II), all of segments I–VII usually but not always with distinct submedian accessory tergal plates. Seta 0-III–VII well developed, normally branched, largest on segments IV and V, inserted close to posterolateral edge of tergal plate; 1-I–VII fully palmate with moderately pigmented leaflets, leaflets with distinct shoulders and long slender filaments (blades and shoulders narrower on segment I), blades usually with distal patch of darker pigment near shoulder (Fig. 2D); 3-I–III,V,VI fairly long, single, 3-IV usually double or triple (2–4), 3-VII triple; 9-I,II inserted anteromesal to seta 7; 2-II,III,VII branched, 2-IV–VI single (2-VI rarely double); seta 6-IV–VI branched at base, triple (2 of 16 seta 6-VI 4-branched). Pecten plate with 12–16 spines (usually 14 or 15) with basal denticles on dorsal side, long spines usually at each end with several interspersed among short spines. Seta 1-S large, with 7–11 branches (usually with 7, 8 or 9 branches); 2-S small, with 7–10(8) branches. Saddle moderately to darkly pigmented, length

0.20–0.24 mm (mean = 0.22 mm). Seta 1-X single, simple, inserted on saddle, much longer than saddle; 2-X with 19–22(19) branches, most basal branches on dorsal side of rachis, relatively straight, with fine tapering tips; 3-X with 8–13 (often 11 or 12) long, thick, slightly curved, apically hooked branches; 4-X (ventral brush) with 9 offset pairs of setae, longest branches on anterior side of main stems. Dorsal and ventral anal papillae equal in length, short, shorter than saddle.

Molecular analysis.—Among the nine mosquitoes available for study (five females, specimen nos. CH6–1, −3, −5, −7, and −13; four males, specimen nos. CH6–8, −9, −10, and −12), DNA from individual −5 consistently failed to amplify by PCR, indicating poor DNA extraction. Of the remaining eight specimens, six yielded good sequence traces for the D3 region, all of which matched *An. minimus* species A at all of the five nucleotide positions that distinguish it from *An. minimus* species C (Sharpe et al. 2000, Chen et al. 2002, 2003). Sequencing of the COII fragment proved more difficult and only three sequences were obtained, of which only one was of good quality. Sequence from this individual, CH6–3, matched *An. minimus* A at the four sites with fixed differences between species A and species C in Thailand, in the region of 294 bp sequenced (Sharpe et al. 2000). These four bases all match *An. minimus* species A sequences obtained in China (Chen et al. 2002, 2003). Based on the availability of COII and D3 sequences, specimen number CH6–3 was selected as the neotype for *An. minimus* (see above and below). The sequences obtained for this specimen are deposited in GenBank under accession numbers AM039906 (D3) and AM039907 (COII).

Systematics.—*Anopheles minimus* is the nominotypical member of a sibling species complex comprised of three or possibly four genetic species (see introduction) that are virtually isomorphic; hence, unambiguous diagnosis of this species is only achievable by means of genetic and molecular markers (see above). Having said this, *An. minimus* is partially, albeit dubiously, distinguished from species C of the complex by the absence of a humeral pale spot (HP) on the wings. Green et al. (1990) found that this spot was absent in 95% of *An. minimus* females (as species A) but was missing in only 22% of species C females from Kanchanaburi Province in western Thailand. Similarly, Sharpe (1997) recorded the absence of a HP spot in 91% of *An. minimus* (as species A) but only 37% of species C collected at the same locality (Ban Phu Rat) visited by Green et al. (1990). Chen et al. (2002) noted the absence of this spot in a comparable percentage of *An. minimus* females (92.7%, as species A) from southern China, but it was missing in a significantly greater number of species C females (84.4%). Finally, Van Bortel et al. (1999) observed an even higher degree of similarity between the two species in northern Vietnam where 99% of *An. minimus* (as form I) and 91.8% of species C (as form II) lacked HP spots. From these observations, it is obvious that the presence or absence of HP spots cannot be used to identify or distinguish the two species with any degree of confidence. Van Bortel et al. (1999) and Chen et al. (2002) also recorded the presence/absence of a presector pale spot on the wings of males and females and showed that this character is even less reliable for distinguishing the two species.

Anopheles minimus is very similar to three other species of the Myzomyia Series that occur within its range of distribution in the Oriental Region, i.e. *An. aconitus, An. fluviatilis,* and *An. varuna.* As pointed out by Harrison (1980), no morphological characters are completely reliable for distinguishing the adults of these species. Furthermore, the adults of *An. pampanai* are also often misidentified as *An. minimus* because the distinguishing features of the wings are not easily discerned. Consequently, adults of *An. minimus* (as well as those of other members of the Minimus Com-

plex) cannot be distinguished from the adults of these species with certainty without associated larval and pupal exuviae. Distinguishing morphological features are provided in the identification keys of Harrison (1980). However, because of the uncertainties associated with morphological differentiation, the various types of molecular assays developed by Sharpe et al. (1999), Van Bortel et al. (2000), Kengne et al (2001), Phuc et al. (2003), and Garros et al. (2004) should be used for the unequivocal identification of *An. minimus* (= their *An. minimus* species A).

The names of four nominal species (one with an unjustified replacement name, see synonymy) are currently regarded as junior synonyms of *An. minimus,* but only one of these, *formosaensis I* Tsuzuki, is certain to denote the same biological species. Tsuzuki (1902) described *An. formosaensis I* from adult mosquitoes collected at an undisclosed location on the island of Taiwan (locality 3 in Fig. 1C). Evidence shows that species C of the Minimus Complex does not extend into eastern areas of China, and only *An. minimus* occurs in Taiwan (Chen et al. 2002).

The syntype specimens (adults) of *An. vincenti* were collected at Van Linh in the former French protectorate of Tonkin (Laveran 1901), which in 1946 formed the northern part of Vietnam bordering on China (locality 2 in Fig. 1C). Electrophoretic studies of the octanol dehydrogenase (*Odh*) enzyme locus indicate that *An. minimus* and species C both occur at this locality (Nguyen Duc Manh, personal communication). Whether some or all of the syntypes of *An. vincenti* are conspecific with either *An. minimus* or species C is unanswerable. As indicated above, adults of these species are indistinguishable in northern Vietnam, and molecular methods are unlikely to be useful in resolving the identity of the syntypes because they are mounted in balsam on a single microscope slide (Harrison 1980). For these reasons, *vincenti* should remain in synonymy with *An. minimus* until the application of this name is resolved.

Anopheles christophersi and variety *alboapicalis* were described by Theobald (1902 and 1910, respectively) from specimens collected in localities of northeastern India that reside in present-day West Bengal (localities 4 and 5, respectively, in Fig. 1C). Before Garros et al. (2005b) and Chen et al. (2006) showed that *An. fluviatilis* species S was conspecific with *An. minimus* species C, only *An. minimus* (as species A) was known to occur in India. Species C is now known to have a wide distribution in the northern states of Madhya Pradesh, Orissa, and Rajasthan. Although this species (as either *minimus* C or *fluviatilis* S) has not been reported from the more easterly state of West Bengal, its possible occurrence in this geographic area cannot be ruled out. The location of the type of *An. fluviatilis* is unknown (Knight and Stone 1977, Harrison 1980), and it probably does not exist. James (1902) apparently described this species from several places in India, including localities in West Bengal where type specimens of *An. christophersi* and variety *alboapicalis* originated. Hence, it is possible that one or more of the names *fluviatilis, christophersi*, or *alboapicalis* may apply to either *An. minimus* or species C.

Fixing the identity of *An. minimus* by neotype designation does not resolve the taxonomic identity of species C, i.e., whether it should be denoted by an available name or recognized as a new species. In contrast, there is clearly no available name for species E of the complex from Ishigaki Island, Japan, and it should be formally described and named as a species new to science.

Material examined.—Nine individually reared specimens (5 females, 4 males), each with associated larval and pupal exuviae on microscope slides. Neotype, female (CH6–3), CHINA: New Territories, Hong Kong, Ham Hang Mei (22° 13′ N, 114° 13′ E), 11 April 2002, stream margin in full sun with dead leaves (L. Rueda & J. Pecor). Other

specimens, same data as neotype: 4 females (CH6–1, CH6–5, CH6–7, CH6–13); 4 males (CH6–8, CH6–9, CH6–10, CH6–12). The specimens are deposited in the USNM. Sequences for DNA extracted from a hind-leg taken from the neotype are deposited in GenBank: accession no. AM039906 for D3, AM039907 for COII.

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